מכון ויצמן למדע
THE WEIZMANN INSTITUTE OF SCIENCE
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DEPARTMENT OF GENETICS המחלקה לגנטיקה

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Dear Dan,

I hope you had a pleasant trip back to the U.S., and that the shock of re-entry was not too great.

Here are some interim results (see attached graphs, tables, etc.):-

(A) Size of the synthetic RNA. The gel electrophoresis patterns seem nice and clean. Most of the c-RNAs are fairly homogeneous at 1S to 2S (100,000 to 200,000M.W?). There is some big stuff (or double stranded RNA?) at the origin, in each case.

(B) Hybridization. Experiment 1. I first checked the hybridizing capacity of the "denatured" SV40 DNA II preps that you left me. From the data in experiment 1, it seems clear that they were incompletely denatured (both the unlabeled and ^{14}C-labeled preps). It is interesting to note from reactions 7 - 12 that whilst the hybridizing capacity jumps 8-fold after the second denaturation, the amount of DNA absorbed to the filters (from the ^{14}C - counts) is approximately the same in each case. It, therefore, seems likely that much of the SV40 DNA II without the second denaturation (though absorbed to the filters) was not available for hybridization. For the filters in the following experiments, I therefore combined your SV40 DNA II + ^{14}C-SV40 DNA II (to make 100 cpm/μg) dialysed the mixture against SSC/100 and denatured by boiling for 15 mins.

Experiment 2. There was only 0.4 ml of the late SV40 RNA (from BSC1 cells) left and as you can see I never reached saturation. Nevertheless, with the remaining 0.3 ml of late SV40 RNA (!) I did experiment 3. It seems to suggest that the cold synthetic RNA completely competes with "late" SV40 - cell RNA. Of course, this must be repeated with saturating amounts of the late RNA. I am preparing another batch and will be ready to do the repeat competition experiment next week.

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We are all well. The Egyptians got a lovely bashing at the canal the other day. So we may expect some quiet and tranquility there until they recover. But the big news at the moment is your men on the moon and Israel is full of admiration.

With greetings to Jo-an and your boys from myself, Audrey and our boys.

Shalom,

Ernest,

Ernest Winocour.

Exp. 1. Capacity of SV40 DNA II (D.N. Preps), with and without Further Denaturation to Hybridize with ^{3}H-SV40 c RNA

Filter No.	DNA on Filter	cpm ^{3}H-SV40 cRNA bound per filter	cpm ^{14}C-SV40 DNA absorbed per filter
1	0.25 μg SV40 DNA II without further treatment	594	-
2	ditto	580	-
3	0.25 μg SV40 DNA II boiled 15 mins in SSC/100	4400	-
4	ditto	3375	-
5	None (blank filter)	128	-
6	ditto	119	-
7	0.28 μg ^{14}C-SV40 DNA II without further treatment	300	212
8	ditto	325	175
9	0.28 μg ^{14}C-SV40 DNA II boiled 15 mins in SSC/100	2549	190
10	ditto	2700	240
11	None (blank filter)	83	9
12	ditto	114	9

Preparation of filters 1,2,7,8; 0.02 ml of SV40 DNA II (25 μg/ml) or 0.02 ml of ^{14}C-SV40 DNA II (28 μg/ml) diluted in 4 ml of 6 x SSC and each loaded on to 2 filters. Dried and heated 80°C, 4 hrs.

Preparation of filters 3,4,9,10; 0.02 ml of SV40 DNA II (25 μg/ml) or 0.02 ml of ^{14}C-SV40 DNA II (28 μg/ml) added to 2 ml of SSC/100, boiled 15 mins and fast cooled. Salt concentration was then adjusted to 6 x SSC and each was then loaded on to 2 filters. Dried and heated heated 80°C, 4 hours.

Filters 1 -6 were then placed in one scintillation vial containing 8 ml of ^{3}H-SV40 cRNA (20,000 cpm/ml in 2 x SSC). Similarly, filters 7-12 were placed in a second vial containing 8 ml of ^{3}H-SV40 cRNA at the same concentration. Incubation was at 65°C for 20 hours. The filters were then washed, exposed to RNAase (20 μg/ml in 2 x SSC; 1 hour at room temperature) and again washed with 2 x SSC at room temperature.

Exp. 2. Hybridization Between a Fixed Amount of ^{14}C-SV40 DNA and Increasing Amounts of ^{3}H-RNA (late) from SV40-infected BSC-1 cells

^{3}H-RNA in solution	RNA input (cpm x 10^{-4})	cpm / SV40 filter ^{3}H	cpm / SV40 filter ^{14}C	cpm / blank filter ^{3}H	cpm / blank filter ^{14}C
SV40 infected cell RNA (late)	60	420	29	19	8
	12	140	34	12	8
	3	37	33	5	8
Uninfected cell RNA	60	7	33	7	8

Late RNA from SV40-infected BS C-1 cell, labeled 24-96 hours post-infection. ^{14}C-SV40 DNA (100 cpm/μg) (D. N. preps) dialyzed SSC/100, denatured (2nd time) by boiling for 15 minutes and loaded on to filters at 1 μg/filter. After drying + baking at 80°C, Smaller filters (9 mm) were punched out of the center of the loaded filters. Each hybridization reaction contained one ^{14}C-SV40 DNA filter (0.3 μg SV40 DNA) one blank filter and 0.2 ml of the RNA in 2 x SSC. Incubation was at 65°C for 20 hours. 2 ml of 2 x SSC was then added to each vial and the filters washed by "vortexing". (This washing procedure was repeated 3 times). 2 ml of 2 X SSC containing 20 μg/ml RNAase were then added to each vial . After 1 hour at room temperature the filters were then washed as before by "vortexing"; the filters were removed from the vials and washed on both sides by suction filtration with 20 ml portions of 2 x SSC. Machine backgrounds not subtracted.

Exp. 3. "Competition" Between Late ^{3}H-SV40 RNA (from infected BSC1 cells) and Unlabelled Synthetic SV40 c-RNA.

First Incubation (11 hours at 65°C)	Second Incubation (20 hours at 65°C)	cpm / SV40 filter	
		^{3}H	^{14}C
0.1 ml 2 x SSC	0.165 ml of ^{3}H-SV40 RNA (late)	531	25
0.1 ml SV40 synthetic RNA (SV-X) (14.7 μg)	ditto	28	15
0.1 ml PV synthetic RNA (PV-F) (10.7 μg)	ditto	434	22

In the second incubation, the concentration of ^{3}H-SV40 RNA (late) was 9×10^{5} cpm per 0.265 ml reaction volume. This is slightly higher than the concentration of ^{3}H-RNA used in experiment 2, but is still below the saturation level for the amount of ^{14}C-SV40 DNA on the filter (approx. 0.2 μg). Hybridization and RNAase conditions were exactly as in experiment 2. Machine backgrounds have not been subtracted.